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Comparative effect of oral administration and topical application of alcoholic extract of *Terminalia arjuna* bark on incision and excision wounds in rats

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Abstract

The effects of 50% ethanolic extract of the bark *Terminalia arjuna* and tannins isolated from the bark were studied for wound healing activity in incision and excision wound models, after oral or topical application in form of a hydrogel. The findings revealed a statistically significant increase in the tensile strength of the incision wounds and increase in the percent reduction in wound size of excision wounds as compared to control. However, the topical treatment with tannins was found to be superior in both incision and excision wound studies. The estimated increase in hydroxyproline content of the granulation tissue of the excision wounds indicated rapid collagen turnover thus, leading to rapid healing of the wounds.

Keywords: *Terminalia arjuna*; Incision wounds; Excision wounds; Hydroxyproline

1. Introduction

The bark of the tree *Terminalia arjuna* W et A (Combretaceae) commonly known as Arjuna bark, indigenous to India and Bangladesh, has been used in Indian system

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medicine (ISM) for at least 3000 years. It is reputed as cardiotonic and also possesses hypotensive and hypolipidemic activity [1]. Its' use in wound healing has been mentioned by Sushruta in Sushruta Samhita [2], however, the claim has yet not been scientifically validated. The ISM mentions different plants possessing wound healing property which has been attributed to the tannins, as they have astringent effects which help to draw the tissues together and also improve resistance to infection [3,4].

Reports also exist with respect to the presence of tannins in Arjuna bark [4]. Hence, the present study was undertaken with the aim of exploring the wound healing activity of the 50% alcoholic extract of Arjuna bark and the tannins present therein in experimental wounds in rats.

2. Experimental

2.1. Plant material

T. ariuna bark collected in the month of October 1999 were procured from Dadar Pharmacy, Mumbai and authenticated at St. Blatter's Herbarium, St. Xavier's College, Mumbai, India.

2.2. Extraction and isolation

Powdered bark were Soxhlet-extracted with 50% EtOH. The alcoholic extract was evaporated in vacuo and the residue (yield: 35% w/w) was subjected to the tests of Kokate [5] and Trease and Evans [6]. The phytochemical screening revealed the presence of tannins, saponins and reducing sugars.

Tannins were isolated by treatment of the extract with a lead acetate solution; the precipitate of lead tannate by treatment with H_2S released tannins which were collected in an alcoholic solution and estimated gravimetrically (yield: 12% w/w) [7].

2.3. Animals

Male Wistar rats weighing 200–250 g were used. They were kept in a standard environmental condition and fed with rodent diet and water ad libitum.

2.4. Incision wound

A 6-cm long incision was made through the shaved skin and cutaneous muscles of the rat and incision was closed with interrupted sutures with stitches 0.5 cm apart [8]. Animals were divided in six groups of six animals each and treated for 10 days as follows:

Group I was used as control, groups II and III were treated orally with 150 and 300 mg kg^{-1} day^{-1} extract, group IV was treated orally with 10 mg kg^{-1} day^{-1} of tannins, groups V and VI were treated topically with 1.5 and 0.1% w/w dissolved

in Pluronic F.127 of extract or tannin, respectively. The sutures were removed on the 8th post-wound day and the tensile strength of the 10-day-old wound was measured.

2.5. Excision wound

Full thickness excision wound was made on the shaved back of the rat by removing a 4 cm^2 piece of skin and the day on which wound was made was considered as day 0 [9].

Animals divided in six groups were treated as described above. The percent wound closure was recorded on day 4, 8, 12 and 16 and the wound area was traced and measured planimetrically. The actual value was converted into percent value taking the size of the wound at the time of wounding as 100%. The granulation tissues were removed on 4th, 8th and 10th post-wound days and analysed for hydroxyproline content [10].

2.6. Statistical analysis

The data obtained were subjected to statistical analysis using Dunnett's '*t*'-test.

3. Results

A significant increase in the tensile strength of the wounds was observed after oral administration as well as topical treatment with 50% ethanolic extract of the *T. arjuna* bark. Also, the tannins isolated from the bark of the plant showed a significant activity. Comparing both the doses of 50% ethanolic extract, the tensile strength of 437 g seen with 300 mg kg^{-1} day^{-1} was found to be comparable to that of topical treatment with 1.5% w/w of extract in the form of hydrogel prepared using PF-127 [11]. Orally administered tannins at a dose of 10 mg kg^{-1} day^{-1} showed results similar to that of 150 mg kg^{-1} day^{-1} of 50% ethanolic extract. However, animals treated with topical application of 0.1% tannins in the form of a hydrogel prepared with PF-127 revealed the highest tensile strength of 462 g (Table 1).

The rate of healing of excision wound was faster in animals treated orally or topically with ethanolic extract compared to the control group. There was no statistically significant difference in the rate of wound healing in animals subjected to oral treatment with a higher dose of 50% ethanolic extract (300 mg kg^{-1} day^{-1}) or topical application of the extract. The oral treatment with tannins was found to be comparable with that of 50% ethanolic extract (150 mg kg^{-1} day^{-1}). However, the fastest rate of healing was revealed in animals treated topically with tannins as compared to other treatment groups as evident from the data on the days required for complete epithelisation (Table 2). Moreover, the profound granulation tissue formation and a wavy nature of the edges of the wounds seen on topical application of the tannins indicated a rapid healing process.

Table 1
Effect of 50% ethanolic extract and tannins of *T. arjuna* bark on tensile strength of incision wounds[a]

Treatment groups	Tensile strength (g)
Control	306 ± 5.5
Alcoholic extract (150 mg/kg[b])	415 ± 4.5*
Alcoholic extract (300 mg/kg[b])	437 ± 6.3*
Tannin (10 mg/kg[b])	415 ± 3.3*
Alcoholic extract (1.5% w/w[c])	440 ± 3.7*
Tannin (0.1% w/w[c])	462 ± 2.4*
Pluronic F.127 (30% w/w)	316.66 ± 4.5

Values are mean ± S.E., $*P < 0.01$ vs. control; Dunnett's '*t*'-test.
[a] $N = 6$, [b]orally, [c]topically.

A significant increase in the hydroxyproline content of the granulation tissue was observed in animals subjected to oral administration of the extract as compared to control indicative of collagen turnover which may contributing to the wound healing capability of the extract (Table 2) [10].

4. Discussion

The results of the present study revealed that both the 50% ethanolic extract of *T. arjuna* and tannins isolated from the bark have significant wound healing activity in both incision as well as excision wound models. The oral treatment of the ethanolic extract was found to be comparable in activity with the topical treatment of the 50% ethanolic extract. However, the topical treatment with tannins was found to be superior to all other treatments as evidenced by lesser number of days required for complete epithelisation of excision wounds and increased tensile strength of incision wounds.

Wound healing is a fundamental response to tissue injury that results in restoration of tissue integrity. This is mainly achieved by the synthesis of the connective tissue matrix. Collagen is a major protein of the extracellular matrix and is the component that ultimately contributes to wound strength. Breakdown of collagen liberates free hydroxyproline and its peptides. Measurement of this hydroxyproline, therefore, has been used as an index of collagen turnover. The increased hydroxyproline content of the excision wounds has indicated faster collagen turnover leading to rapid healing with concurrent increase in the tensile strength of the treated wounds [12].

Similar findings have been reported with the extracts of the plants containing tannins by earlier workers [13,14]. But so far tannins have not been isolated and tested for wound healing property. However, our results revealed that tannins are one of the important phytoconstituents responsible for wound healing mainly due to their astringent and antimicrobial property. Moreover, tannins when delivered topically in the form of a hydrogel showed excellent wound healing properties.

Hence, it can be inferred that the wound healing activity of the bark of the plant *T. arjuna* is due to its high tannin content, which seems to be responsible for wound contraction and increased rate of epithelisation.

Table 2
Effect of 50% ethanolic extract and tannins of *T. arjuna* bark on %wound closure and hydroxyproline content of excision wounds[a]

Groups	Day 4	Day 8	Day 12	Day 16	Epithelisation period (days)	Hydroxyproline (μg/100 mg)
Control	21.60 ± 0.62	51.40 ± 1.08	71.40 ± 0.61	85.20 ± 0.55	24	1770 ± 23.96
Alcoholic extract (150 mg/kg[b])	35.80 ± 0.37*	77.90 ± 0.67*	93.30 ± 0.45*	98.80 ± 0.54*	18	2146 ± 23.50*
Alcoholic extract (300 mg/kg[b])	41.60 ± 0.33*	88.50 ± 0.58*	98.90 ± 0.61*	100*	14	2224 ± 37.70*
Tannin (10 mg/kg[b])	31.90 ± 0.55*	81.30 ± 0.47*	92.90 ± 0.71*	98.20 ± 0.15*	20	2156 ± 63.30*
Alcoholic extract (1.5% w/w[c])	40.90 ± 0.76*	89.90 ± 0.53*	98.90 ± 0.43*	100*	14	
Tannin (0.1%w/w[c])	59.00 ± 0.47*	97.90 ± 0.36*	100*	100*	8	

Values are mean ± S.E., *$P < 0.01$ vs. control; Dunnett's '*t*'-test.
[a] $N = 6$, [b]per oral, [c]topical.

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